

B1  
acid residues of SEQ ID NO: 2, provided that the protein and the protein variant give rise to tumor antigen peptides that are capable of binding to an HLA antigen and being recognized by cytotoxic T lymphocytes; --

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Please replace the paragraph beginning on page 6, line 24 with the following amended paragraph:

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B2  
--(2) A DNA consisting of a base sequence shown in SEQ ID NO: 1, or a DNA variant that hybridizes to the DNA under a stringent condition, provided that a protein produced and expressed by the DNA or the DNA variant gives rise to tumor antigen peptides that are capable of binding to an HLA antigen and being recognized by cytotoxic T lymphocytes; --

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Please replace the paragraph beginning on page 11, line 5 with the following amended paragraph:

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B3  
--The DNAs of the present invention encode novel tumor antigen proteins, and specific examples of the DNAs include a DNA encoding SART-3 protein consisting of an amino acid sequence shown in SEQ ID NO: 2, or a protein variant consisting of an amino acid sequence containing substitution, deletion, and/or addition of one or more amino acid residues of the amino acid sequence of SART-3, provided that the protein and the protein variant give rise to tumor antigen peptides that are capable of binding to an HLA antigen and being recognized by cytotoxic T lymphocytes; or a DNA of SART-3 consisting of a base sequence shown in SEQ ID NO: 1, or a DNA variant that hybridizes to the DNA of SART-3 under a stringent condition, provided that a protein produced and expressed by the DNA and the DNA variant gives rise to tumor antigen

B3  
peptides that are capable of binding to an HLA antigen and being recognized by cytotoxic T lymphocytes. The DNA of the present invention is further described hereinafter following the order established above.--

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Please replace the paragraph beginning on page 11, line 21 with the following amended paragraph:

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--1) DNA encoding SART-3

B4  
“DNA encoding a protein consisting of an amino acid sequence shown in SEQ ID NO: 2” and “a DNA consisting of a base sequence shown in SEQ ID NO: 1” among the DNAs described above refers to a DNA encoding tumor antigen protein SART-3 of the present invention. The DNA may be cloned in accordance with the process described in Examples hereinafter. Further, the cloning of the DNA may be also conducted by, for example, screening a cDNA library derived from cell lines such as esophageal cancer cell line KE-4 (FERM BP-5955) using an appropriate portion of the base sequence disclosed in GenBank Accession No. D63879 or shown in SEQ ID NO: 1 in the present specification as a probe for hybridization or a PCR primer. It would be ready for those skilled in the art to achieve such cloning in accordance with Molecular Cloning 2nd Edt. Cold Spring Harbor Laboratory Press (1989), for example.--

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Please replace the paragraph beginning on page 12, line 25 with the following amended paragraph:

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--3) DNA that hybridizes to the DNA of SART-3 under a stringent condition

B5  
"DNA variant that hybridizes to the DNA of SART-3 under a stringent condition" among the DNAs described above refers to a DNA that hybridizes to human SART-3 cDNA consisting of the base sequence shown in SEQ ID NO: 1 under a stringent condition, including SART-3 DNAs from all of vertebrate such as rat and mouse, and DNAs encoding a partial protein of SART-3. --

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Please replace the paragraph beginning on page 13, line 14 with the following amended paragraph:

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B6  
--The DNA variants are cloned by diverse processes such as hybridization to the DNA shown in SEQ ID NO: 1. Particular procedures for the processes such as production of cDNA library, hybridization, selection of positive colony, and determination of base sequence are well-known, and may be conducted consulting Molecular Cloning as shown above. Probes useful for the hybridization includes a DNA comprising a base sequence described in SEQ ID NO: 1.--

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Please replace the paragraph beginning on page 17, line 12 with the following amended paragraph:

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B7  
--In the invention, the term "protein" refers to a protein encoded by the various DNAs of the present invention as described above, which has an ability as tumor antigen protein to give rise to tumor antigen peptides via intracellular degradation that are capable of binding to an HLA antigen and being recognized by CTLs. Specific examples of the proteins include SART-3 comprising an amino acid sequence shown in SEQ ID NO: 2. The proteins of the present

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invention may be produced in large scale using the DNA of the present invention  
as described above. --

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Please replace the paragraph beginning on page 22, line 5 with the following amended  
paragraph:

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--It is easy to select peptide portions involved in such motifs from the  
amino acid sequence of the protein of the present invention. Such peptide  
portions involved in the above motif structures can be easily selected by  
inspecting the amino acid sequence of tumor antigen protein SART-3 (SEQ ID  
NO: 2). Further, it is easy to select any sequence expected to be capable of  
binding to HLA antigens by search on internet as shown above. Tumor antigen  
peptides of the present invention can be identified by synthesizing candidate  
peptides thus selected according to the method described above and conducting an  
assay for determining whether or not a complex between the candidate peptide  
and an HLA antigen is recognized by CTL, in other words, whether or not a  
candidate peptide has an activity as a tumor antigen peptide. --

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Please replace the paragraph beginning on page 25, line 7 with the following amended  
paragraph:

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--Accordingly, HLA-A24- and HLA-A2-restricted tumor antigen peptides  
among the tumor antigen peptides of the present invention are exemplified by  
those tumor antigen peptides that are partial peptides involved in such motif  
structures or structures expected to be capable of binding to the HLAs in the

B9 amino acid sequence of SART-3 shown in SEQ ID NO: 2 and that are capable of binding to respective HLA antigens and being recognized by CTLs.—

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Please replace the paragraph beginning on page 48, line 19 with the following amended paragraph:

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--Determination of Base Sequence of Tumor Antigen Protein Gene

B10 The base sequence of the DNA of tumor antigen protein SART-3 as obtained in Example 3 was determined using DyeDeoxy Terminator Cycle Sequencing kit (Perkin-Elmer). The base sequence thus determined is shown in SEQ ID NO: 1. The full-length of the cDNA was 3798 base pairs. Amino acid sequence (963 amino acids) encoded by the base sequence of SEQ ID NO: 1 is shown in SEQ ID NO: 2. Comparison of the base sequence shown in SEQ ID NO: 1 to known sequences using GenBank data base revealed that the base sequence of tumor antigen protein SART-3 has a novel base sequence that is different from gene KIAA0156 registered at GenBank under Accession No. D63879 in terms of a single base (at position 108 of KIAA0156), which function has not been demonstrated.--

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Please replace the paragraph beginning on page 49, line 10 with the following amended paragraph:

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--Selection of Candidate Peptides

B11 There are certain rules (motifs) in the sequences of antigen peptides that should be bound and presented by HLA antigens. Regarding the motif for HLA-A24, it is known that in the sequence of peptides consisting of 8 to 11 amino

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acids, the amino acid at position 2 is tyrosine, phenylalanine, methionine, or tryptophan, and the amino acid at the C-terminus is phenylalanine, tryptophan, leucine, isoleucine, or methionine (*Immunogenetics*, 41:178, 1995; *J. Immunol.*, 152:3913, 1994; *J. Immunol.*, 155:4307, 1994). According to the motifs, peptide portions consisting of 8 to 11 peptides having the above motifs were selected from the amino acid sequence of tumor antigen protein SART-3 shown in SEQ ID NO: 2. Those examples of the selected peptides are shown in SEQ ID NOs: 3-24.

These peptides were synthesized at Biologica Co. by the Fmoc method.--

The paragraph on page 51, line 21 with the following amended paragraph:

B12  
--To this peptide resin, 2 ml of Reagent K (the solution of 5% phenol, 5% thioanisole, 5% H<sub>2</sub>O, and 2.5% ethanedithiol in TFA) was added and the mixture was allowed to react for 2.5 hours at room temperature. While cooling with ice, 10 ml of diethyl ether was added to the reaction, the mixture was stirred for 10 minutes, filtered, and washed with 10 ml of diethyl ether. To the filter cake, 10 ml of aqueous acetic acid was added, and the mixture was stirred for 30 minutes. The resin was then filtered, and washed with 4 ml of aqueous acetic acid. After lyophilizing the filtrate and the wash, the crude peptide obtained was dissolved in aqueous acetic acid, and injected into a reverse phase packing material, YMC-PACK ODS-A column (30  $\phi$  x 250 mm) that had been pre-equilibrated with 0.1% aqueous TFA. The column was washed with 0.1% aqueous TFA, and elution at a flow rate of 7 ml/min was then conducted, while increasing the concentration of

acetonitrile up to 25% over 180 minutes. The eluate was monitored by A 220 nm.

The fractions containing the desired product were combined together and lyophilized to obtain 31.0 mg of Val-Tyr-Asp-Tyr-Asn-Cys-His-Val-Asp-Leu(SEQ ID NO:3).--

Please replace the paragraph beginning on page 52, line 13 with the following amended paragraph:

--The peptide obtained, Val-Tyr-Asp-Tyr-Asn-Cys-His-Val-Asp-Leu(SEQ ID NO:3), had a retention time of 19.3 minutes in an analysis using a reverse phase packing material, YMC-PACK ODS-AM column (4.6  $\phi$  x 250 mm) eluted with a linear gradient of acetonitrile concentration from 16 to 46% containing 0.1% TFA, and the results of amino acid analysis (Cys being not detected) and mass spectrometry of the product were consistent with the theoretical values.--

Please replace the paragraph beginning on page 53, line 21 with the following amended paragraph:

--(2) Synthesis of SART-3 "172-181" Leu-Phe-Glu-Lys-Ala-Val-Lys-Asp-Tyr-Ile (SEQ ID NO: 4)

According to a similar manner to that described in above (1), using 100 mg of Fmoc-Ile-Alko Resin (0.41mmol/g, 100-200mesh), Fmoc-Tyr(tBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Phe-OH, and Fmoc-Leu-OH

B14

were coupled in order, and the product was then deprotected. The crude peptide obtained was dissolved in aqueous acetic acid and injected into a reverse phase packing material YMC-PACK ODS-A column (30  $\phi$  x 250 mm) that has been pre-equilibrated with 0.1% aqueous TFA. The column was washed with 0.1% aqueous TFA, and the elution at a flow rate of 7 ml/min was then conducted, while increasing the concentration of acetonitrile up to 30% over 300 minutes. The eluate was monitored by A 220 nm. The fractions containing the desired product were combined together and lyophilized to obtain 66.3 mg of Leu-Phe-Glu-Lys-Ala-Val-Lys-Asp-Tyr-Ile(SEQ ID NO:4). --

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Please replace the paragraph beginning on page 54, line 12 with the following amended paragraph:

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B15

--The peptide obtained, Leu-Phe-Glu-Lys-Ala-Val-Lys-Asp-Tyr-Ile(SEQ ID NO:4), had a retention time of 23.8 minutes in an analysis using a reverse phase packing material YMC-PACK ODS-AM column (4.6  $\phi$  x 250 mm) eluted with a linear gradient of acetonitrile concentration from 12 to 42% containing 0.1% TFA, and the results of amino acid analysis and mass spectrometry of the product were consistent with the theoretical values.--

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Please replace the paragraph beginning on page 55, line 10 with the following amended paragraph:



--(3) Synthesis of SART-3 "284-292" Asn-Tyr-Asn-Lys-Ala-Leu-Gln-Gln-Leu  
(SEQ ID NO: 5)

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According to a similar manner to that described in above (1), using 100 mg of Fmoc-Leu-Alko Resin, Fmoc-Gln-OH, Fmoc-Gln-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asn-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asn-OH were coupled in order, and the product was then deprotected. The crude peptide obtained was dissolved in aqueous acetic acid and injected into a reverse phase packing material YMC-PACK ODS-A column (30  $\phi$  x 250 mm) that has been pre-equilibrated with 0.1% aqueous TFA. The column was washed with 0.1% aqueous TFA, and the elution at a flow rate of 7 ml/min was then conducted, while increasing the concentration of acetonitrile up to 30% over 300 minutes. The eluate was monitored by A 220 nm. The fractions containing the desired product were combined together and lyophilized to obtain 25.0 mg of Asn-Tyr-Asn-Lys-Ala-Leu-Gln-Gln-Leu(SEQ ID NO:5).--

Please replace the paragraph beginning on page 55, line 26 with the following amended paragraph:

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--The peptide obtained, Asn-Tyr-Asn-Lys-Ala-Leu-Gln-Gln-Leu(SEQ ID NO:5), had a retention time of 19.0 minutes in an analysis using a reverse phase packing material YMC-PACK ODS-AM column (4.6  $\phi$  x 250 mm) eluted with a linear gradient of acetonitrile concentration from 12 to 42% containing 0.1%

217  
TFA, and the results of amino acid analysis and mass spectrometry of the product were consistent with the theoretical values.--

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Please replace the paragraph beginning on page 56, line 21 with the following amended paragraph:

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--(4) Synthesis of SART-3 "315-323" Ala-Tyr-Ile-Asp-Phe-Glu-Met-Lys-Ile

(SEQ ID NO: 6)

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According to a similar manner to that described in above (1), using 100 mg of Fmoc-Ile-Alko Resin (0.62mmol/g, 100-200mesh), Fmoc-Lys(Boc)-OH, Fmoc-Met-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Phe-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Ile-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Ala-OH were coupled in order, and the product was then deprotected. The crude peptide obtained was dissolved in aqueous acetic acid and injected into a reverse phase packing material YMC-PACK ODS-A column (30  $\phi$  x 250 mm) that has been pre-equilibrated with 0.1% aqueous TFA. The column was washed with 0.1% aqueous TFA, and the elution at a flow rate of 7 ml/min was then conducted, while increasing the concentration of acetonitrile up to 40% over 180 minutes. The eluate was monitored by A 220 nm. The fractions containing the desired product were combined together and lyophilized to obtain 15.4 mg of Ala-Tyr-Ile-Asp-Phe-Glu-Met-Lys-Ile(SEQ ID NO:6). --

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Please replace the paragraph beginning on page 57, line 11 with the following amended paragraph:

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--The peptide obtained, Ala-Tyr-Ile-Asp-Phe-Glu-Met-Lys-Ile(SEQ ID NO:6), had a retention time of 19.6 minutes in an analysis using a reverse phase packing material YMC-PACK ODS-AM column (4.6  $\phi$  x 250 mm) eluted with a linear gradient of acetonitrile concentration from 21 to 51% containing 0.1% TFA, and the results of amino acid analysis (Met being not detected) and mass spectrometry of the product were consistent with the theoretical values.--

Please replace the paragraph beginning on page 58, line 7 with the following amended paragraph:

--(5) Synthesis of SART-3 "416-425" Asp-Tyr-Val-Glu-Ile-Trp-Gln-Ala-Tyr-Leu (SEQ ID NO: 7)

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According to a similar manner to that described in above (1), using 100 mg of Fmoc-Leu-Alko Resin, Fmoc-Tyr(tBu)-OH, Fmoc-Ala-OH, Fmoc-Gln-OH, Fmoc-Trp(Boc)-OH, Fmoc-Ile-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Val-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH were coupled in order, and the product was then deprotected. The crude peptide obtained was dissolved in aqueous acetic acid and injected into a reverse phase packing material YMC-PACK ODS-A column (30  $\phi$  x 250 mm) that has been pre-equilibrated with 0.1% aqueous TFA. The column was washed with 0.1% aqueous TFA, and the elution at a flow rate of 7 ml/min was then conducted, while increasing the concentration

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of acetonitrile up to 35% over 180 minutes. The eluate was monitored by A 220 nm. The fractions containing the desired product were combined together and lyophilized to obtain 18.9 mg of Asp-Tyr-Val-Glu-Ile-Trp-Gln-Ala-Tyr-Leu(SEQ ID NO:7).--

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Please replace the paragraph beginning on page 58, line 23 with the following amended paragraph:

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18 21  
--The peptide obtained, Asp-Tyr-Val-Glu-Ile-Trp-Gln-Ala-Tyr-Leu(SEQ ID NO:7), had a retention time of 20.5 minutes in an analysis using a reverse phase packing material YMC-PACK ODS-AM column (4.6  $\phi$  x 250 mm) eluted with a linear gradient of acetonitrile concentration from 25 to 55% containing 0.1% TFA, and the results of amino acid analysis (Trp being not detected) and mass spectrometry of the product were consistent with the theoretical values.--

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Please replace the paragraph beginning on page 59, line 19 with the following amended paragraph:

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--(6) Synthesis of SART-3 "426-434" Asp-Tyr-Leu-Arg-Arg-Arg-Val-Asp-Phe (SEQ ID NO: 8)

According to a similar manner to that described in above (1), using 100 mg of Fmoc-Phe-Alko Resin (0.72mmol/g, 100-200mesh), Fmoc-Asp(OtBu)-OH, Fmoc-Val-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Leu-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH were coupled in

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B22

order, and the product was then deprotected. The crude peptide obtained was dissolved in aqueous acetic acid and injected into a reverse phase packing material YMC-PACK ODS-A column (30  $\phi$  x 250 mm) that has been pre-equilibrated with 0.1% aqueous TFA. The column was washed with 0.1% aqueous TFA, and the elution at a flow rate of 7 ml/min was then conducted, while increasing the concentration of acetonitrile up to 25% over 240 minutes. The eluate was monitored by A 220 nm. The fractions containing the desired product were combined together and lyophilized to obtain 34.0 mg of Asp-Tyr-Leu-Arg-Arg-Arg-Val-Asp-Phe(SEQ ID NO:8).--

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Please replace the paragraph beginning on page 60, line 10 with the following amended paragraph:

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--The peptide obtained, Asp-Tyr-Leu-Arg-Arg-Arg-Val-Asp-Phe(SEQ ID NO:8), had a retention time of 20.1 minutes in an analysis using a reverse phase packing material YMC-PACK ODS-AM column (4.6  $\phi$  x 250 mm) eluted with a linear gradient of acetonitrile concentration from 12 to 42% containing 0.1% TFA, and the results of amino acid analysis and mass spectrometry of the product were consistent with the theoretical values. --

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Please replace the paragraph beginning on page 61, line 5 with the following amended paragraph:

--(7) Synthesis of SART-3 "448-456" Ala-Phe-Thr-Arg-Ala-Leu-Glu-Tyr-Leu  
(SEQ ID NO: 9)

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According to a similar manner to that described in above (1), using 100 mg of Fmoc-Leu-Alko Resin, Fmoc-Tyr(tBu)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Phe-OH, and Fmoc-Ala-OH were coupled in order, and the product was then deprotected. The crude peptide obtained was dissolved in aqueous acetic acid and injected into a reverse phase packing material YMC-PACK ODS-A column (30  $\phi$  x 250 mm) that has been pre-equilibrated with 0.1% aqueous TFA. The column was washed with 0.1% aqueous TFA, and the elution at a flow rate of 7 ml/min was then conducted, while increasing the concentration of acetonitrile up to 30% over 240 minutes. The eluate was monitored by A 220 nm. The fractions containing the desired product were combined together and lyophilized to obtain 22.8 mg of Ala-Phe-Thr-Arg-Ala-Leu-Glu-Tyr-Leu(SEQ ID NO:9).--

Please replace the paragraph beginning on page 61, line 21 with the following amended paragraph:

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--The peptide obtained, Ala-Phe-Thr-Arg-Ala-Leu-Glu-Tyr-Leu(SEQ ID NO:9), had a retention time of 18.1 minutes in an analysis using a reverse phase packing material YMC-PACK ODS-AM column (4.6  $\phi$  x 250 mm) eluted with a linear gradient of acetonitrile concentration from 20 to 50% containing

B23  
0.1% TFA, and the results of amino acid analysis and mass spectrometry of the product were consistent with the theoretical values. --

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Please replace the paragraph beginning on page 66, line 4 with the following amended paragraph:

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--Identification of HLA-A2-Restricted Tumor Antigen Peptides

B23  
On the basis of the amino acid sequence of tumor antigen protein SART-3 shown in SEQ ID NO: 2, peptide sequences consisting of nine or ten amino acid residues that were expected to be capable of binding to HLA-A0201 were searched on internet using a software of NIH BIMAS ([http://bimas.dcrt.nih.gov/molbio/hla\\_bind/](http://bimas.dcrt.nih.gov/molbio/hla_bind/)). Those examples of the searched peptides are shown in SEQ ID NOS 25-52. These peptides were synthesized at Biologica Co. by the Fmoc method. --

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Please replace the Sequence Listing filed February 28, 2001 located immediately after the abstract with substitute Sequence Listing enclosed herewith.

IN THE CLAIMS

Please amend the claims as follows:

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B27  
(amended)1. A DNA encoding a protein consisting of an amino acid sequence shown in SEQ ID NO: 2, or a protein variant consisting of an amino acid sequence containing substitution,